

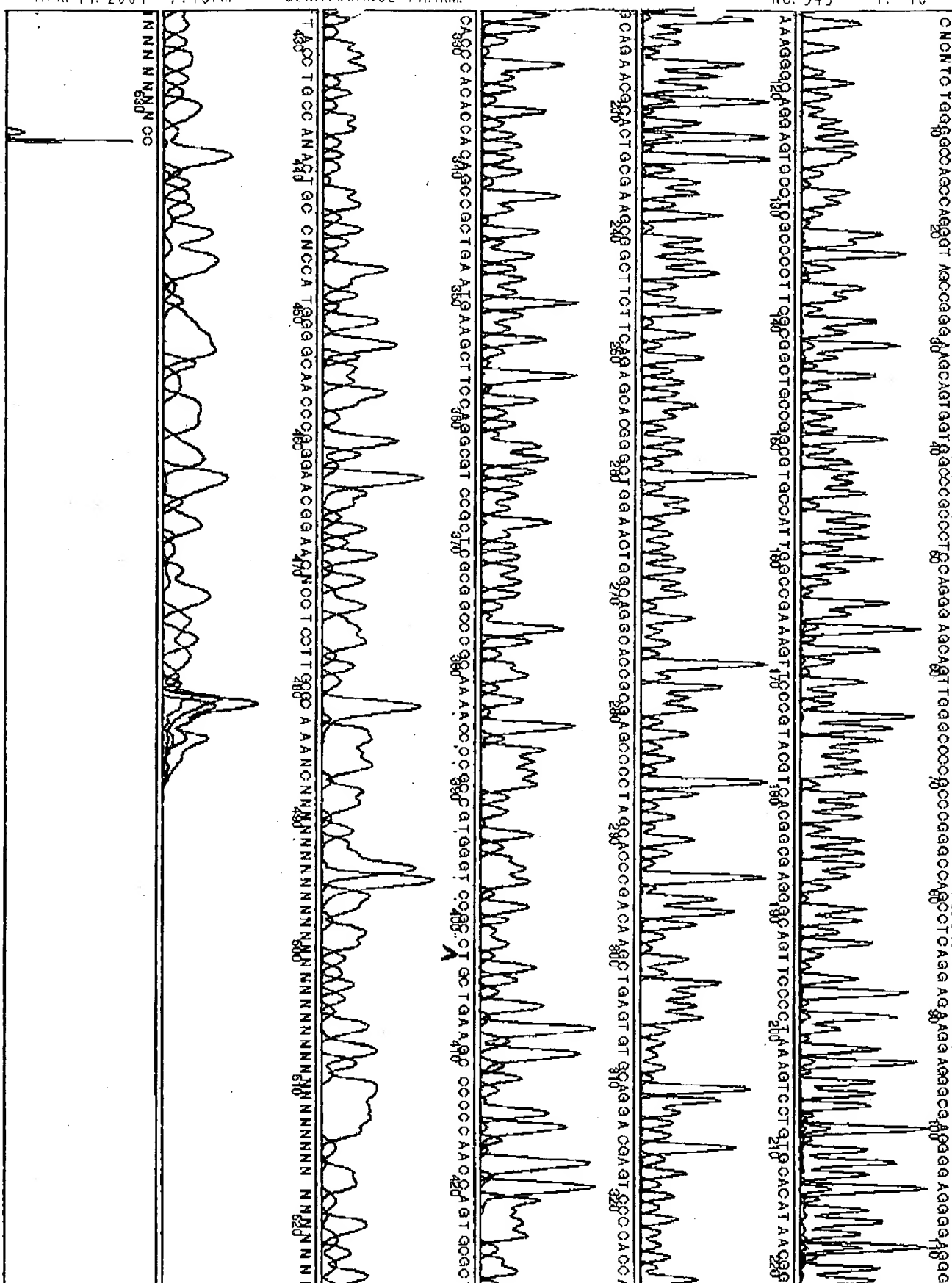


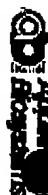
**ABI Applied Biosystems**  
 Model 373A  
 Version 2.0.18  
 Bb78 20  
 Dye Terminator(Any Primer)  
 Lane 20  
 Signal: G:757 A:1184 T:300 C:377

INST FILE 808296  
088-1369  
MCGRAW-A-2F-1

Tue, Mar 5, 1986 8:51 PM  
X: 0 to 6634 Y: 0 to 1600  
Spacing: 10.40

Page 1 of 1  
A3-F





Model 373A  
Version 2.0.1S

B5/9 18  
Dye Terminator{AnyPrimer}  
Lane 16  
Signal: G:1186 A:1244 T:5

INST FILE 808296  
086-1364  
MCGRAW-A-2/R-

Tue, Mar 5, 1996 8:51 PM  
X: 0 to 6640 Y: 0 to 1600  
Spacing: 10:38

Page 1 of 1

15-2

CAC AAGNA GGGCT GGCGTTGGGAGT TGGCCCATGCGCGCAAT CTGCGAGGTAAAGCGACTGCTGCGAGGCGCGCTCAGTAAGCGCGNCCGACGCGAGGAGCTCTGCGAGCGCG

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AAGCAGGCTGAAGAAGCTCATTCAGGCGCTATGATGATGAGATATGATGAGAGATCTGATGCTAACAACATCGAGCTGATGGGATGACTAGGAGGATGCGAGATAGCTACCAAGT

GCACCCCGAT 280 CTCTGAAGAAGCCGCGTTCCGATGCGT TCGGCCGCTA TGTGCACAGGA 280 TTAGGGGAAGT GCCCTCGCCGATGCGT ACGGGAACCTTTCGCCCA 300

TAGCA CACCAGC AGC<sup>870</sup> OGGGA AAGGCGA AAGCATC<sup>960</sup> CTCCCT TTTCCCTCCCCT<sup>880</sup> GGCCTGCCCT<sup>930</sup> TCCTCTCTCCT<sup>400</sup> AAAGT GGCCTGAGGAGGAC<sup>420</sup> CAACT GCT

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Model 373A  
Version 2.0.1S

b5/9 18  
 Dyeterminator(AnyPrimer)  
 Lane 18  
 Signal: G:1128 A:1741 T:5

INST FILE 808298  
098-1367  
MCGRAW--DWMF-1

Tue, Mar 5, 1986 8:51 PM  
X: 0 to 6615 Y: 0 to 1600  
Spacing: 10.43

Page 1

**Signal: G:1128 A:1741 T:517 C:580**

**MOGRAW--DWMF-1**

DUM-F

CNCNCTC T aaagacaaaccacaggat AACCCAGG A AACAAT GATgcccccccccT TCACAGGAAAGCAATTGCCCCCGGCCAACAGGC CCAACCTCAGG ANA AGG AAAAG CGNAGAG KAAAGTGGG

AAAGGGAACGATGGCTCACCCCCTT<sub>180</sub>GCGAGCTGCCTGCGCATTGCCCATTTGGCCGAAGA<sub>190</sub>TCCCGTAACGTACACACGAGAGGCGAGTTCCCGTAAAGTCCTGT<sub>200</sub>GCACATAAAGGACC

CAGAAAGGACTGCGA<sup>230</sup>AGGAGCTTCT<sup>260</sup>TGAGAGACACGAG<sup>280</sup>CTGGAAC<sup>270</sup>TGAGGACACCG<sup>290</sup>GAAGCCCT<sup>320</sup>ATGACCCGAC<sup>300</sup>ATGAGTGT<sup>310</sup>AGGACGAA<sup>320</sup>TTCCCAACCA<sup>320</sup>

30' G A C A C C A G A G C C G C T G A A T G A A G C T T C G A N A G C A T C C G G T O G C G A C C G G C A A A A A C C C G G C C A T G G A T C G A C C T G C T G A G C C C C C C A A G G A A T G C C A T

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Model 373A  
Version 2.0.1S

BB/914  
DyeTert  
Lane 14  
Signal: C

Dye Terminator{AnyPrimer}

Signal: G:1181 A:1187 T:548 C:530

INST FILE 808296  
096-1362  
MCGRAW--DW/M/R-1

Tue, Mar 5, 1996 8:51 PM  
X: 0 to 6650 Y: 0 to 1600  
Spacing: 10.47

Page 1

DM-R

CAC AAGAA GGGCT GCGGT TGGGG ATGGCGCGAGT CTGGCGGT AAAGCGCT GAGCTGGGGCGCGCT CAAAGCGAG ACCGACCGCGCGCT CTGGCGCG

[illegible]

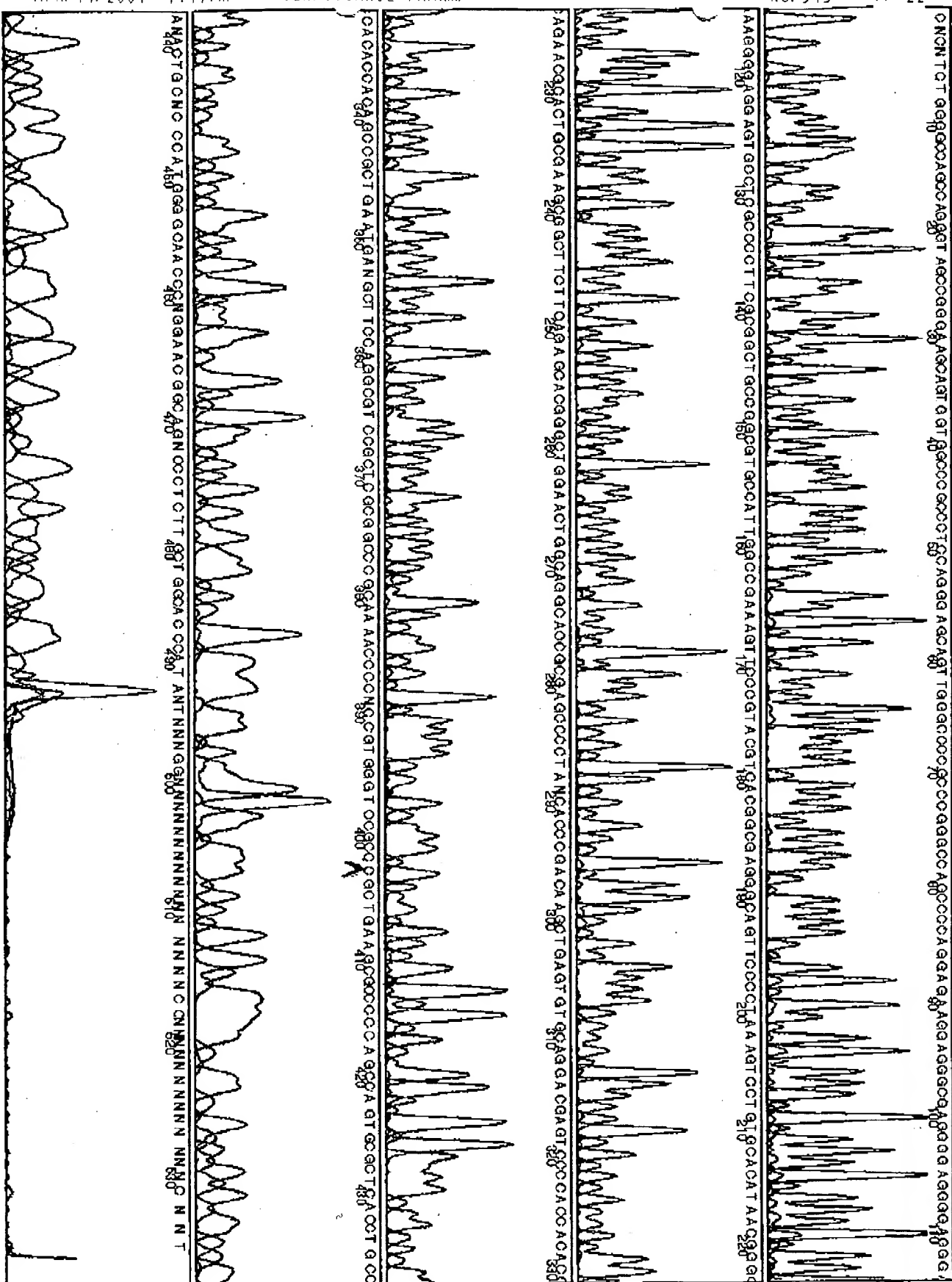
225 C A G C C G G T G T O T G A A G A A G C C C G C T T C G G A G T G C A T T O T G C C C A T T A 250  
 255 G C A G A G G A G C T T T A G G G A A G C T T G C C C T C G C C A T G A C A T A C C G G A A C T T 280  
 285 G G C C

A T A G G A C G C C A G G A A G G C G A A G G A G C A G T C T C C C C T T T C C C T C C C C T G C C C T C G C C C A T G T C T C C T G A A G C T A G C C G N G A G C C G N A G C T A C T G C  
 360 370 380 390 400 410 420


[illegible]

Model 373A  
Version 2.0.1SBioss UR  
Dye Terminator/Any Primer  
Lane 8  
Signal: G:406 A:399 T:97 C:121INST FILE 808296  
096-2859  
MCGRAW/HAG/F1Thu, May 9, 1996 4:57 PM  
X: 0 to 6472 Y: 0 to 1600  
Spacing: 10.26

Page 1



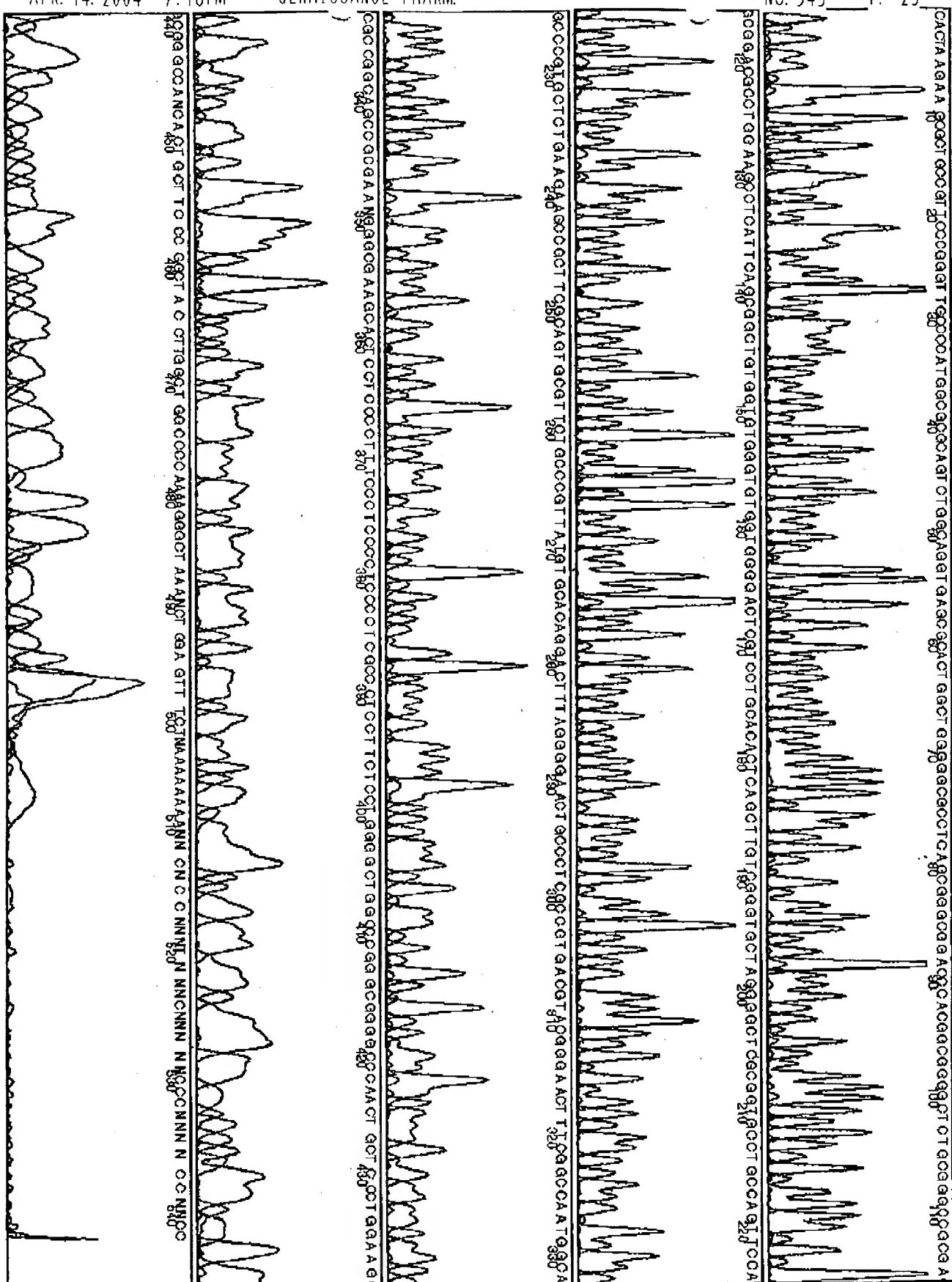


 <b>Applied Biosystems</b> Model 373A Version 2.0.1S	Hs33b Uy DyeTerminator(AmyPrimer) Lane 9 Signal: G:315 A:191 T:85 C:85
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INST FILE 808296  
098-2860  
MCGRAW/HAS/R1

Thu, May 9, 1996 4:57 PM  
X: 0 to 6452 Y: 0 to 1600  
Spacing: 10.31

Page 1



PCR (B2AR)

1/26/96

\* Made new set of primers for B2AR 5' flanking region. ~~Best~~  
Product should include the CRE + short ORF. Primers  
were chosen = MacVector

Blood samples are being obtained from pts in the UC asthma  
clinic by Melanie Meyers.

DNA samples prepared by Liz Donnelly using ~~Amplify~~ +/m  
Diana kits. Samples are numbered as received (A1, A2, etc.)

- Set up PCR rxn using new primers + made up 96ul master mix.

10ul buffer II

6ul 25mM MgCl<sub>2</sub>

0.8ul 25mM dNTP

0.5ul 100uM forward primer (B2AR-F1)

0.5ul 100uM reverse primer (B2AR-R1)

77.7ul dH<sub>2</sub>O

0.5ul amplitag

- digest 24ul of master mix into 2 PCR tubes

- add 1ul template DNA

- overlay = 1 drop mineral oil

- perform PCR in thermal cycler = denat gradient made on

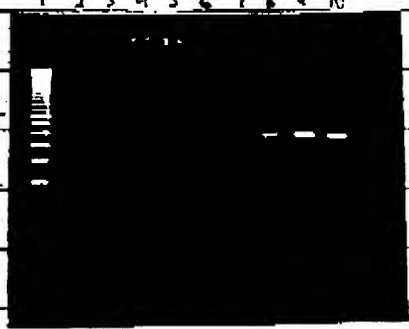
94°C x 2 min

94°C x 30 sec / 64°, 63°, 60°, or 58°C x 30 sec / 72°C x 30 sec => 35 cycles

72°C x 7 min

- run 10ul of PCR rxn on TBE minigel

1/26/96 (cont)

	1	2	3	4	5	6	7	8	9	10
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size	100									

1 - 100 bp ladder

6 - blank

2 - A1 (Amplify) 64°C

7 - A1 (Amplify) 64°C

3 - " 62°C

8 - " 62°C

4 - " 60°C

9 - " 60°C

5 - " 58°C

10 - " 58°C

No product seen = DNA isolated by Amplify kit. Nice band seen = DNA from Qiagen kit but expected size should be 508 bp. The band present appears to be < 400 bp.

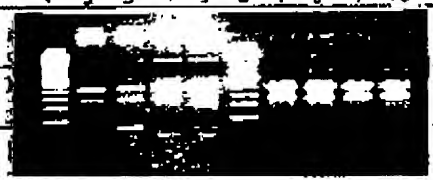
Repeat PCR with following modification:

- add 73.7 ul dH<sub>2</sub>O

- digest 23 ul of master mix into PCR tubes

- add 2 ul template DNA

- change cycling temp to Amplify cycle to 56°, 54°, 52° + 50° C; change cycling temp for Qiagen cycle to 58°, 56°, 54°, 52° C.

	1	2	3	4	5	6	7	8	9	10
1 - 100 bp ladder										
2 - A1 (Amplify) 58°C										
3 - " 54°C										
4 - " 52°C										
5 - " 50°C										
6 - 100 bp ladder										
7 - A1 (Qiagen) 58°C										
8 - " 56°C										
9 - " 54°C										
10 - " 52°C										

Now have bands in all samples. However, uppermost band present in both samples sets appears to be < 500 bp, still smaller than expected.



B2AR PCR (5 $\mu$ l)

Set up master mix for four (4) 25 $\mu$ l PCR rxns:

2 $\mu$ l	template DNA (DWM)
1.5 $\mu$ l	forward primer
1.5 $\mu$ l	reverse primer
20 $\mu$ l	5X buffer (buffer A from Phastagene PCR optimization kit)
10 $\mu$ l	dUTP <sub>s</sub> (2.5mM) (from optimization kit)
65 $\mu$ l	dH <sub>2</sub> O
0.8 $\mu$ l	Tag
100 $\mu$ l	total

- aliquot 25  $\mu$ l of master mix into 4 PCR rxn tubes

- overlay  $\approx$  1 drop of mineral oil

- PCR cycle	98°C	2 min	} 30 cycles
	98°C	30 sec	
	56° 54° 52° or 50°	30 sec	
	72°C	30 sec	
	72°C	7 min	

- remove 10 $\mu$ l aliquot + run on minigel

#1 - 100 bp ladder

#2 - 56°C

#3 - 54°C

#4 - ~~56~~ 52°C

#5 - 50°C

